Ratio of neutrophil to lymphocyte Counts in Crimean Congo Hemorrhagic Fever

Kırım Kongo Kanamalı Ateş Hastalığında Nötrofil Lenfosit Oranı

NLR in CCHF Patients

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Özet

Bakteriyel ve viral enfeksiyonlarda yeni belirteçlerin klinik uygulamada kullanımını onların maliyetleri sınırlandırmaktadır. Bu çalışmada kırım kongo kanamalı ateşi (KKKA) hastalığında nötrofil lenfosit oranının (NLO) değerlendirilmesi amaçlandı. NLO kanama gözlenmeyen 77 hasta ve kanama gözlenen 27 hasta olmak üzere toplam 104 KKKA hastasında ve 61 sağlıklı yetişkinlerde ölçüldü. Hastaların NLO değerleri, hastaneye başvurunun birinci ve üçüncü günde ölçüldü. Medyan NLO değeri kontrol grubunda 4.10 iken hasta grubunun 1. gününde 4,02 ve 3. gününde 2,02 olarak tespit edildi.3. gündeki NLO değerleri (p <0.01) kanama gözlenen grupta 1,16 (0,05-3,40) ve kanama gözlenmeyen grupta 2,32 (0,35-19,45) olarak bulundu. NLO değeri kene ile temasın 3. gününden itibaren, hastalığın klinik seyri ve tedavinin belirlenmesinde yol gösterici bir işaret gibi görünmektedir.

Anahtar Kelimeler

KKHA; Nötrofil Lenfosit Oranı; Acil Servis

Abstract

The implementation of new markers of bacterial and viral infection into clinical practice is hindered by their costs. In this study we assessed the potential use of the neutrophil to lymphocyte count ratio (NLCR) in crimean congo hemorraghic fever (CCHF). NLCR values were measured in total 104 patients with CCHF with 77 patients in whom bleeding was observed and 27 patients without bleeding observed and in 61 healthy adults. NLCR values of patients were measured in the first and 3rd days in which the patients admitted to the hospital. Median NLCR value was found as 4.02 in the patient group on the 1st day and 2.02 on the 3rd day, while this value was 4.10 in the healthy adults. NLCR values of the 3rd day were found as 1.16 (0.05-3.40) in the patients with bleeding and 2.32 (0.35-19.45) in the patients without bleeding (p<0.01). NLCR value seems to be a marker guiding in determination of the clinical course and treatment from the 3rd day of the contact with ticks.

Keywords

CCHF; Ratio of Neutrophil to Lymphocyte Counts; Emergency Service

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Introduction

Crimean-congo hemorrhagic fever (CCHF) is a zoonotic viral disease caused by a tick- borne virus of the Nairovirus genus in the Bunyaviridae family [1,2]. CCHF has a fatality rate of 3-30% of cases and the pathogenesis of the disease is not well understood [3]. CCHF characteristically occurs in humans via a tick bite or from contact with the blood or tissues of infected livestocks [4]. The incubation period for CCHF ranges from 2 to 12 days after the tick bite. This period ranges from 3 to 10 days in nosocomial cases [5,6]. The hemorrhagic period develops rapidly and usually begins between the fifth and seventh days of disease [7]. Patients may show signs of progressive hemorrhagic diathesis, such as petechiae, mucous membranes hemorrhage, conjunctival hemorrhage, nosebleed, hemoptysis, hematuria, hematemesis, and melena [7-9]. It has been reported that mononuclear phagocytes, hepatocytes, and endothelial cells are major targets of CCHF virus infection [10]. Despite increasing knowledge about hemorrhagic fever viruses, little is known about the pathogenesis of CCHF [2].

Absolute lymphocytopenia (lymphocyte count < 1.0 × 109/L) in the course of the immune response to systemic infection is a relatively unknown phenomenon to physicians [11-13]. Initially, lymphocytopenia has been described in case reports concerning infectious emergencies such as toxic shock syndrome [14]. Later, Zahorec demonstrated in a prospective longitudinal observational study the correlation between the severity of the clinical course and lymphocytopenia in patients treated for severe sepsis and septic shock in an oncologic intensive care unit (ICU) [15].

Crimean-Congo hemorrhagic fever disease is a viral infection. However, it shows a course similar to sepsis with development of DIC manifestation, especially in severe cases. Therefore, in this study we aimed to measure the prognostic value of NLCR in the diagnosis and treatment of Crimean Congo hemorrhagic fever disease which is a viral systemic disease and manifests with bleeding.

Material and Method

One hundred four consecutive patients (40 male and 64 female, mean age 45±19 years) with a laboratory confirmed diagnosis of CCHF were retrospektif enrolled in the study over a period of two years (April 2011 and October 2013). Sixty one age and sex matched healthy volunteers were included in the study as controls. Patients using anticoagulants and platelet aggregation inhibitors and people with chronic diseases such as chronic obstructive pulmonary disease, hematological disorders, malignancies and hepatobiliary disease were excluded. Twenty three patients were excluded with respect to these exclusion criteria. All confirmed CCHF patients were classified into two groups based on disease severity (bleeding observed in 77 patients and bleeding was not observed in 27 patients). Patients with at least one of the followings were considered severe cases: somnolence, melena, activated partial thromboplastin time (APTT) ≥ 60s and thrombocyte count ≤ 20x109 cell/l [25]. Blood counts were analyzed on the 1st and 3rd days. The aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), prothrombin time (PT), international normalized ratio (INR), aPTT, platelet count, WBC, hemoglobin, lymphocytes, neutrophil and leukocytes were measured in all patients. Mindray Auto Hematology Analyzer BC-6800, Chine was used for the determination of neutrophil count and lymphocytes levels.

Statistical analysis

Statistical analysis was performed using SPSS software, version 15 (SPSS, Chicago, IL). Distribution of continuous variables was tested by the Kolmogorov–Smirnov test. Continuous variables were expressed as means±standard deviation or medians and 25th–75th percentile values (Interquartile range: IQR) (normally and not normally distributed, respectively). Statistical differences among groups were tested by independent –samples Kruskal-Wallis for nonparametric variables, respectively. Univariate linear regression model was used to adjust differences in mortality for age and sex in CCHF patients and controls.

Results

The study group consisted of one hundred four (64 male and 40 female, mean age 45±19 years). There were 61 healthy volunteers (35 male and 26 female, mean age 43±15) in the control group. Gender and age were similar in the CCHF groups and healthy volunteers groups (Table 1).

Table 1. Demographic Characteristics of Crimean Congo Hemorraghic Fever Patients and Control Groups

	Patient Group (n=104)	Control Group (n=61)	p Value
Mean age (year)	45±19	43±15	0.50
Male	64	35	0.62

When the patient and control groups were compared on the first day; a significant difference was observed between the groups in terms of ALT, AST, LDH, leukocytes, neutrophils, platelets, INR, lymphocytes, white blood cells and platelet counts ($p< 0.01$). Of these values, ALT, AST, LDH and INR increased, while a decrease was found in terms of leukocytes, neutrophils, platelets, lymphocytes, and platelet counts.

While NLCR was insignificant in the patient group compared to the controls on the first day [4.02 (0.07-27.6) vs 4.10 (2.17-8.11) respectively; $P> 0.05$], this value was the only significant biomarker on the 3rd day [4.02 (0.07-27.6) vs 2.02 (0.05-19.05) respectively; $P< 0.01$] (Table 2).

When the first and 3rd days were compared in the patient group;

Table 2. Laboratory Characteristics of Crimean Congo Hemorraghic Fever Patients and Control Groups

	1. day	3. day	Control group
ALT (U/L)	64±44*¶	115±94*	18±5
AST (U/L)	119±90*¶	195±177*	18±4
LDH	435±245*¶	534±302*	130±14
Lökosit	3.3±1.9*	3.5±2.1*	6.1±1.2
Neutrophil	2.23±1.55*¶	1.68±1.08*	5.36±0.92
Plt	85±46*	77±48*	281±67
INR	1.04±0.08*	1.02±0.05*	0.92±0.08
Lymphocit	0.76±0.43*¶	1.06±0.69*	1.38±0.33
NLCR	4.02 (0.07-27.6) ¶	2.02 (0.05-19.5)*	4.10 (2.17-8.11)

AST; Aspartate aminotransferase, ALT;alanine aminotransferase, LDH ; lactate dehydrogenase, PT; prothrombin time, INR; international normalized ratio, Plt; platelet count, wbc; white blood cell, NLCR; neutrophil to lymphocyte count ratio. *P < 0.01 vs patients with control group. ¶P < 0.01 vs 1 ve 3.day.

Table 3. Characteristics laboratory values of the patients classified according to the severity of disease.

	Non-severe (n:58)	Severe (n:46)	p Value
ALT, U/L	56,62±40,89	72,71±47,61	0,067
AST, U/L	101,31±82,26	140,58±96,47	0,027
LDH, U/L	360 (112-921)	531 (151-933)	0,001
Platelets, x103/mm3	93,43±39,31	73,76±50,86	0,028
Fibrinogen	267,46±36,30	242,78±45,96	0,003
NLCR	3,36 (0,42-14,85)	4,85 (0,07-27,63)	0,084
Neutrophils, x103/mL	1,95 (0,44-5,87)	2,58 (0,40-5,85)	0,040
Lymphocytes, x103/mL	0,78±0,40	0,74±0,46	0,627
Hemoglobin, g/dL	11,86±1,21	11,96±1,15	0,654
Leukocyte, x103/mL	3310,15 (1500-7640)	3378,26 (600-6541)	0,858
INR	1,01±0,08	1,05±0,07	0,518
D-dimer,	1114,05 (88-5289)	2821,86 (116-5247)	0,001

AST; Aspartate aminotransferase, ALT;alanine aminotransferase, LDH ; lactate dehydrogenase, PT; prothrombin time, INR; international normalized ratio, Plt; platelet count, wbc; white blood cell, NLCR; neutrophil to lymphocyte count ratio.

Table 4. Characteristics laboratory values of the patients classified according to the mortality of disease.

	Non fetal (N:93)	Fetal (N:11)	P Value
ALT, U/L	60,82±42,85	88,36±52,52	0,052
AST, U/L	110,47±85,64	188,09±104,94	0,007
LDH, U/L	403,04 (112-933)	710,81 (156-929)	0,001
Platelets, x103/mm3	90,53±43,09	35,65±37,35	0,001
Fibrinogen,	262,41±37,77	206,90±49,24	0,001
NLCR	3,85 (0,14-25,21)	5,47 (0,07-27,63)	0,243
Neutrophils, x103/mL	2,01 (0,14-5,87)	4,12 (0,04-5,85)	0,008
Lymphocytes, x103/mL	0,72±0,39	1,11±0,57	0,050
Hemoglobin, g/dL	11,89±1,18	12,04±1,27	0,692
Leukocyte, x103/mL	3179,66 (610-7640)	4698,18 (600-7535)	0,012
INR	1,04±0,08	1,07±0,06	0,291
D-dimer	1630,24 (88-5289)	3891,63 (219-5247)	0,001

AST; Aspartate aminotransferase, ALT;alanine aminotransferase, LDH ; lactate dehydrogenase, PT; prothrombin time, INR; international normalized ratio, Plt; platelet count, wbc; white blood cell, NLCR; neutrophil to lymphocyte count ratio.

a significant difference was found in neutrophil count, lymphocyte count and neutrophil-lymphocyte ratio (P < 0.01). ALT, AST, LDH and lymphocyte count increased, while a decrease was observed in the number of neutrophils, platelets, white blood cell and neutrophil lymphocyte ratio.

When the 3rd day of the patient and control group was compared; ALT, AST, LDH, leukocytes, neutrophils, platelets, INR, the number of lymphocytes, neutrophil to lymphocyte count ratio and platelet white blood cell count showed a significant difference. Of these values, ALT, AST, LDH and INR increased, while kocytes, neutrophils, platelets, the number of lymphocytes, neutrophil to lymphocyte count ratio and platelet white blood cell count decreased .

When the patients having bleeding at anywhere were compared with the patients having not complaints of bleeding; there was a significant difference between both the groups in terms of neutrophil to lymphocyte count ratio. Mean NLCR value was found as 2.51 (0.07-6.26) in the bleeding patients and 4.55 (0.42-27.63) in the non-bleeding patients on the 1st day (p: 0.04). Mean NLCR value was found as 1.16 (0.05-3.40) in the bleeding patients and 2.32 (0.35-19.45) in the non-bleeding patients on the 3rd day (p<0.01).

Patients were classified according to the severity of the disease. Significant differences were observed between severe and non-severe patients with respect to AST, LDH, fibrinogen, neutrophil and d-dimer values. LDH, neutrophil and d-dimer were correlated with the severity of disease in the regression analysis of these significant values.(table 3)

Patients were classified according to the mortality of the disease. Significant differences were observed between severe and non-severe patients with respect to AST, LDH, platelets, fibrinogen, neutrophil leukocytes and d-dimer values. Platelets, fibrinogen, neutrophil leukocytes and d- dimer were correlated with the mortality of disease in the regression analysis of these significant values.(table 4)

Discussion

To the best of our knowledge, this study is the first in the published literature to investigate the predictive value of admission NLRC on the severity of CCHF. We found that NLRC was significantly decreased in CCHF patients. Furthermore even after controlling for confounding parameters we found that less NLRC was strongly associated with disease severity.

Previous studies have reported that decreased levels of thrombocytes and increased levels of white blood cells, AST,ALT, LDH, as well as a prolonged APTT were associated with a poor outcome in CCHF patients [16,17].

Lymphocytopenia has been described in case reports concerning infectious emergencies such as toxic shock syndrome [14]. Later, Zahorec demonstrated in a prospective longitudinal observational study the correlation between the severity of the clinical course and lymphocytopenia in patients treated for severe sepsis and septic shock in an oncologic intensive care unit (ICU) [15].

In study of Inci's leukocytes and platelets values were found in CCHF patients to be significantly lower than the control group [18]. In our study, while AST, LDH neutrophil leukocytes and d-dimer levels were significantly higher in fetal group, platelets and fibrinogen levels were significantly lower.

Any of the following clinical pathologic values during the first 5 days of illness were found to be >90% predictive of fatal outcome in a series of South African CCHF patients: platelet counts <20 × 109/l, ALT >150 U/l, AST >200 U/l, leukocyte counts < 10 × 109/l, fibrinogen <110 mg/dl and aPTT >60 s [17]. In a study from Turkey, higher ALT and AST levels (>700 and >900 IU/l, respectively) were found to have higher sensitivity for severe cases [19]. AST, LDH, platelets, fibrinogen, neutrophil leukocytes and d- dimer were significant in our study.

NLCR might be a finding which could be expected to decrease in CCHF that is a viral disease, but decreases observed in the follow-up suggest that the bleeding may occur. In our study, NLCR began to be significant for CCHF disease compared to the control group on the 3rd day of the onset of clinical complaints. Viral replication is likely to be more common in these patients because of the acute period. Both neutrophils and lymphocytes number and variation of the ratio of these values can give us a suggestion about the clinical course.

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Conflict of interest

No conflict of interest and no funding source to declare.

Ethical approval

The study was performed in accordance with the Declaration of Helsinki for Human Research, and was approved by the institutional ethics committee.

References

1. Turkdogan KA, Karabacak M, Akpinar O, Karahan O, Güven FMK, Engin A et al. The VKORC1 gene homozygous polymorphism is markedly higher in Crimean Congo Hemorrhagic Fever patients. Biomedical Research 2013;24(3):415-20.
2.Whitehouse CA. Crimean-Congo hemorrhagic fever. Antiviral Res 2004;64:145-60.
3.Ergonul O,Celikbas A, Dokuzoguz B, Eren S, Baykam N, Esener H. The characteristics of Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and the impact of oral ribavirin therapy. Clin Infect Dis 2004;39:285-9.
4.Mardani M, Keshtkar-Jahromi M. Crimean–Congo hemorrhagic fever. Arch Iran Med 2007;10(2):204-14.
5. Elaldı N. Epidemiology of Crimean-Congo hemorrhagic fever, Klimik Derg 2004;17:151-5.
6. Swanepoel R, Gill DE, Shepherd AJ, Leman PA,Mynhardt JH, Harvey S. The clinical pathology of Crimean/Congo hemorrhagic fever. Rev Infect Dis 1989;11:794-800.
7. Watts DM, Ksiasek TG, Linthicum KJ, Hoogstraal H. Crimean-Congo Hemorrhagic fever. In T.P. Monath, ed. The arboviruses: epidemiology and ecology, vol.2. CRC, Boca Raton, FL,USA. 1988;177-260.
8. Oldfield EC, Wallace MR, Hyams KC, Yousif AA, Lewis DE, Bourgeois AL. Endemic infectious diseases of the Middle East. Rev Infect Dis 1991;13:199-217.
9. Celikbas A, Ergonul O, Baykam N, Eren S, Esener H, Dokuzoguz B.Analysis of the mortality among the patients with Crimean Congo Hemorrhagic Fever virus infection. 15th European Congress of Clinical Microbiology and Infectious Diseases, April 2-5, 2005, Copenhagen. Clin Microbiol Infect 2005;11:19.
10.Burt FJ, Swanepoel R, Shieh WJ, Smith JF, Leman PA, Greer PW, et al. Immunohistochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. Arch Pathol Lab Med 1997;121:839-46.
11.Wyllie DH, Bowler IC, Peto TE. Bacteraemia prediction in emergency medical admissions: role of C reactive protein. J Clin Pathol 2005;58:352-6.
12. Wyllie DH, Bowler IC, Peto TE. Relation between lymphopenia and bacteraemia in UK adults with medical emergencies. J Clin Pathol 2004;57:950-5.
13. Hawkins CA, Collignon P, Adams DN, Bowden FJ, Cook MC. Profound lymphopenia and bacteraemia. Intern Med J 2006;36:385-8.
14. Galus MA, Stern J. Extreme lymphocytopenia associated with toxic shock syndrome. J Intern Med 1998;244:351-4.
15. Zahorec R. Ratio of neutrophil to lymphocyte counts rapid and simple parameter of systemic inflammatiion and stress in critically ill. Bratisl Lek Listy 2001;102:5-14.
16. Schnittler HJ, Feldman H. Viral hemorrhagic fever a vasculer disease? Thromb Haemost 2003;89:967-72.
17. Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean Congo hemorrhagic fever. Rev Infect Dis 1989;11:794-800.
18. Inci A. Increased Mean Platelet Volume in Patients with Crimean Congo Hemorrhagic Fever. J Clin Anal Med 2014; DOI: 10.4328/JCAM.2271.
19. O. Ergonul, A. Celikbas, N. Baykam, S. Eren, B. Dokuzoguz. Analysis of risk factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. Clin Microbiol Infect, 12 (2006), pp. 551–4.

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